MATERIALS AND METHODS FOR DETECTING VAGINAL EPITHELIAL CELLS

GOVERNMENT SUPPORT

This invention was made with government support under 2012-DN-BX-K018 awarded by National Institute of Justice. The government has certain rights in the invention.

The Sequence Listing for this application is labeled "SeqList-22Dec16-ST25.txt", which was created on Dec. 22, 2016, and is 4 KB. The entire content is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

The current methods used in forensic sciences for detecting vaginal epithelial cells in forensic samples are time consuming and presumptive. These methods are based on histological staining of glycogen-rich cells as vaginal cells. Typically a sample collected from a crime scene is used for DNA extraction and a separate portion of the sample is used for serology. Histological staining requires that cells collected from the crime scene are fixed to a microscopic slide, stained, dried, and analyzed by a microscopy expert. Moreover, the cells undergoing histological analysis are no longer viable to be used in other methods, for example, DNA extraction and analysis.

Further, the currently used methods based on histological staining of cells are prone to false results. For example, false negatives occur because the glycogen content of vaginal cells varies depending on the menstrual cycle and reproductive age. False positives can occur due the fact that buccal and urogenital skin cells (even from males) can have high content in glycogen and get stained.

Other methods currently used rely on the quantification of certain RNAs as a product of cell-specific gene expression. Gene expression at the transcription level is quantifiable by the levels of messenger RNA (mRNA) present in a specific type of cells or at the post-transcription level that occurs through micro RNA (miRNA) presence in specific tissues. Certain methods practiced to quantify mRNA or miRNA are capillary electrophoresis and high-resolution melt analysis. The use of RNA requires normalization of transcript levels with those of a housekeeping gene in the same sample. Often the normalization has to be performed by quantifying RNA levels prior to cDNA synthesis. Also, these methods do not discriminate between human and bacterial RNA, which is commonly present in most samples. Since most transcripts are present at a basal level in body fluids, the quantification of RNA for body fluid identification relies on levels of specific transcripts being higher than a certain threshold in specific cells. Lack of an accurate quantification of RNA in the sample leads to false results since it influences the threshold for the target transcript.

BRIEF SUMMARY OF THE INVENTION

The subject invention provides materials and methods for detecting vaginal epithelial cells in a sample comprising cells and/or body fluids, for example, a forensic sample.

In one embodiment, the level of methylation at the PFN3A locus in the genetic material isolated from the sample is used to detect and/or quantify vaginal epithelial cells in a sample. In another embodiment, the level of methylation at the PFN3A locus in the genetic material isolated from a sample is determined by pyrosequencing using specific primers described herein.

A further embodiment of the invention provides a method for determining the level of methylation at the PFN3A locus in the genetic material isolated from a cell, for example, a cell suspected to be a vaginal epithelial cell that is isolated from a forensic sample.

Kits containing primers and reagents for carrying out the methods disclosed herein are also provided.

Assays for determining the level of methylation at the PFN3A locus in the genetic material isolated from a sample are also provided. In certain embodiments, the assays comprise pyrosequencing using specific primers described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 provides a graph showing mean percent of methylation for samples of blood (n=8), saliva (n=11), semen (n=12) and vaginal epithelia (n=10) with 50 ng input to bisulfite. * indicates CpG positions where the difference in methylation levels is not statistically significant (p<0.05) between vaginal epithelia and blood. For each CpG position indicated on the x-axis, the bars from left to right correspond to blood, saliva, semen, and vaginal epithelial cell samples.

FIG. 2 provides a graph showing mean percent of methylation for vaginal epithelia samples (n=6) with different input of DNA. * indicates CpG positions and nanograms of DNA that present methylation levels with statistically significant differences (p<0.05) between groups. For each CpG position indicated on the x-axis, the bars from left to right correspond to 50 ng, 10 ng, 5 ng, and 1 ng DNA.

FIG. 3 provides pyrogram showing a sample with 1 ng input to bisulfite. Peak height for the majority of peaks corresponding to single nucleotide dispensations are lower than 20 light units (y-axis) which creates a warning recorded by software.

FIG. 4 provides mean percent of methylation for samples containing different ratios of DNA from blood (B) and vaginal epithelia (VE). With the decrease in the content of DNA from blood compared to vaginal epithelia, a decrease in the percent of methylation is also observed. The average percent methylation for the blood (n=8) and vaginal epithelia (n=11) samples are shown as control. The samples labeled B and VE are the DNA from blood and vaginal epithelia, respectively, used to make the mixture. For each CpG position indicated on the x-axis, the bars from left to right correspond to blood (n=8), B, 75% B:25% VE, 50% B:50% VE, 25% B:75% VE, VE, and vaginal epithelial samples (n=11).

FIG. 5 provides mean percent of methylation for samples containing different ratios of DNA from blood (B) and semen (S). With the decrease in the content of DNA from blood compared to semen, a decrease in the percent of methylation is also observed. The average percent methylation for the blood (n=8) and semen (n=12) samples are shown as control. The samples labeled B and S are the DNA from blood and semen, respectively, used to make the mixture. For each CpG position indicated on the x-axis, the bars from left to right correspond to blood (n=8), B, 75% B:25% S, 50% B:50% S, 25% B:75% S, S, and semen samples (n=12).

FIG. 6 provides mean percent of methylation for samples containing different ratios of DNA from vaginal epithelia (VE) and semen (S). With the decrease in the content of DNA from vaginal epithelia compared to semen, a decrease in the percent of methylation is also observed. The average percent of methylation for the vaginal epithelial cell sample (n=1) and semen (n=12) samples are shown as control. The